Bridging the Gap between Academic and Commercial Biofuel Production: Pilot-scale Processing of Transgenic Energycane for Lipid and Sugar Recovery

Shraddha Maitra[1]* (smaitra@illinois.edu), Dang Viet Cao[2], Baskaran Kannan[2], Hui Liu[3], Fredy Altpeter[2], John Shanklin[3], Vijay Singh[1], and **Stephen P. Long[1]**

[1]University of Illinois at Urbana-Champaign, Urbana, IL; [2]University of Florida, Gainesville, FL; [3]Brookhaven National Laboratory, Upton, NY

https://rogue.illinois.edu/

Project Goals

The main objective of ROGUE (Renewable Oil Generated with Ultra-productive Energycane) project is to engineer the two most productive American crops—energycane and *Miscanthus*—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts.

1. Increasing oil accumulation and targeting this to the mature stem
2. Increasing photosynthetic efficiency to power oil synthesis
3. Multi-gene construct transformation of energycane and Miscanthus
4. Field testing, processing and techno-economic analysis

Abstract

Biofuels provide sustainable alternatives to petroleum-based fuels and lignocellulosic biomass is considered as the source of abundant renewable carbon. Cellulosic sugars and plant oils are particularly valued as they can be converted conveniently to bioethanol, biodiesel, and bio-jet. However, oilseeds that are also part of human food are still used predominantly for biodiesel production. To this end, transgenic bioenergy crops metabolically engineered to produce and accumulate high triacylglyceride (TAG) content have shown immense potential to replace oilseeds (Parajuli et al., 2020; Vanhercke et al., 2019; Zale et al., 2016). In ROGUE, bioenergy crop-energycane has been genetically modified to shift the carbon flux towards synthesis and accumulation of TAG molecules in their vegetative tissues. Transgenic energycane crops can be an excellent source of fermentable sugars and lipids/oil for biofuel production (Fouad et al., 2015). However, after establishing the basic characteristics of transgenic plants under controlled environmental conditions for genetic design and initial processing at bench-scale, it is critical to scale up the processes and test the proof-of-concept at pilot-scale for holistic analysis needed for potential commercialization. Bioprocessing at a pilot-scale provides industrially relevant data and helps identify research gaps for the successful commercialization of the project. Pilot-scale processing helps in getting better estimates for the techno-economical analysis and process simulation.

The transgenic energycane crop was cultivated under field conditions at the University of Florida-IFAS Plant Science Research and Education Unit, FL, USA. Upon harvesting, brown and green leaves were separated from the stem. A total of 223 kg (166 kg stem; 38.6 kg green leaves+tops; 18.3 kg brown leaves) were received at Integrated Bioprocess and Research Laboratory (IBRL), a translational research facility at the University of Illinois, IL, USA. Before harvest, the total TAG content in green leaves, stem, and juice were 3 to 10% (depending on leaf maturity and position), 0.6 to 1.3% (depending on stem maturity and position), and 1.4% per g dry weight, respectively. Transgenic energycane stems (97 kg) were processed in the pilot-scale facility. A combination of pilot-scale continuous hydrothermal pretreatment and disk milling, followed by hydrolysis with commercial cellulases, successfully recovered fermentable sugars and lipids. Chemical-free pretreatment at pilot-scale has shown no detrimental effect on oil recovery or the lipid profile from these transgenic energy crops. Thus, pilot-scale bioprocessing has been successfully established for transgenic energycane to produce fermentable sugars for bioethanol and lipids for biodiesel.

References/Publications

Parajuli, S., Kannan, B., Karan, R., Sanahuja, G., Liu, H., Garcia-Ruiz, E., Kumar, D., Singh, V., Zhao, H., Long, S., Shanklin, J., Altpeter, F., 2020. Towards oilcane: Engineering hyperaccumulation of triacylglycerol into sugarcane stems. GCB Bioenergy 12, 476–490. https://doi.org/10.1111/gcbb.12684

Vanhercke, T., Belide, S., Taylor, M.C., El Tahchy, A., Okada, S., Rolland, V., Liu, Q., Mitchell, M., Shrestha, P., Venables, I., Ma, L., Blundell, C., Mathew, A., Ziolkowski, L., Niesner, N., Hussain, D., Dong, B., Liu, G., Godwin, I.D., Lee, J., Rug, M., Zhou, X.-R., Singh, S.P., Petrie, J.R., 2019. Up-regulation of lipid biosynthesis increases the oil content in leaves of Sorghum bicolor. Plant Biotechnol. J. 17, 220–232. https://doi.org/10.1111/pbi.12959

Zale, J., Jung, J.H., Kim, J.Y., Pathak, B., Karan, R., Liu, H., Chen, X., Wu, H., Candreva, J., Zhai, Z., Shanklin, J., Altpeter, F., 2016. Metabolic engineering of sugarcane to accumulate energy-dense triacylglycerols in vegetative biomass. Plant Biotechnol. J. 14, 661–669. https://doi.org/10.1111/pbi.12411

Fouad, W. M., Hao, W., Xiong, Y., Steeves,C., Sandhu,S. K., Altpeter, F., 2015. Generation of Transgenic Energy Cane Plants with Integration of Minimal Transgene Expression Cassette. Current Pharmaceutical Biotechnology 2015; 16(5). https://doi.org/10.2174/1389201016666150303151559

Funding Statement

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0018254.